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YOLK TRANSPORT IN THE OVARIAN FOLLICLE OF THE HEN (*GALLUS DOMESTICUS*): LIPOPROTEIN-LIKE PARTICLES AT THE PERIPHERY OF THE OOCYTE IN THE RAPID GROWTH PHASE

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SUMMARY

Thin sections of the oocyte periphery and surrounding granulosa layer from 1–5-day preovulatory follicles were examined by transmission electron microscopy. With the use of certain procedures in tissue preparation, notably the tannic acid method, numerous particles in the range of 15–40 nm with a mean diameter of 27 nm were observed both extra- and intracellularly. The particles were abundant in the granulosa basal lamina, in the spaces between the granulosa cells and in the perivitelline space. They appeared to adhere to the oolemma as a continuous double layer which was also observed to line the coated vesicles, 200–350 nm in diameter, invaginating from the oolemma. The layer of particles was not found on the plasma membranes of the granulosa cells, nor were particles present within the cells. In the peripheral cytoplasm of the oocyte the yolk spheres, ranging upwards from 250 nm diameter, were membrane-bound and contained tightly packed particles similar to those on the oolemma. Bodies displaying features intermediate between coated vesicles and yolk spheres suggested that, on entry into the cell, loss of the cytoplasmic coat and obliteration of the vesicular lumen gave rise to nascent yolk spheres which then fused together to form the larger spheres. The extracellular layer, coated vesicles and smaller yolk spheres were absent in oocytes fixed after a 10-min delay.

The evidence indicated that 27-nm particles were transferred from the basal lamina to the oocyte surface via the intergranulosa cell channels, incorporated into the cell by adsorptive endocytosis and then transferred to the yolk spheres with little morphological alteration. The identity of the particles with very low density lipoproteins, the major components of the yolk solids, was discussed.

INTRODUCTION

The yolk of the hen's ovum is derived from components in the blood plasma and it is generally accepted that many of them are incorporated into the growing oocyte as macromolecules by the process of micropinocytosis (Schjeide *et al.* 1970). Most of the yolk consists of lipid in the form of lipoproteins, which separate on centrifugation into a low density (derivative, lipovitellenin) and a high density (lipovitellin) fraction. The 2 fractions account for 66 and 16%, respectively, of the total yolk solids and 93 and 7%, respectively, of the total yolk lipids (Cook, 1968). The lipid is mainly deposited in the final phase of rapid growth (Marza & Marza, 1935; Bellairs, 1964; Mackenzie & Martin, 1967) when the ovarian follicle increases from 0·15 g to 17 g

in the 7 days preceding ovulation (Gilbert, 1971) and the yolk is characterized by a yellow pigmentation.

In the laying bird, the level of plasma very low density lipoprotein (VLDL) is considerably higher than in immature birds and cockerels as a result of hormonal stimulation of VLDL synthesis in the liver (see Tarlow *et al.* 1977). The similarity in protein and lipid composition of plasma VLDL and egg yolk VLDL (Hillyard, White & Pangburn, 1972; Gornall & Kuksis, 1973) and findings on the fate of labelled VLDL following injection into the bloodstream (Holdsworth, Michell & Finean, 1974) have provided evidence in support of the view that this component is transferred to the oocyte in a relatively unchanged form.

Some structural modifications of the ovarian follicle that coincide with the onset of the final growth stage are considered to facilitate the access to and absorption by the oocyte of macromolecular constituents derived from the capillary bed in the theca interna (Wyburn, Aitken & Johnston, 1965; Wyburn, Johnston & Aitken, 1965; Perry, Gilbert & Evans, 1978*a*, *b*). These modifications include an expansion of the intercellular spaces in the granulosa layer surrounding the oocyte and the formation of large coated pits and vesicles at the oocyte surface. Furthermore, the granulosa basal lamina has been found to contain abundant particles, which appear to be similar to plasma VLDL (Evans, Perry & Gilbert, 1979).

We have recently succeeded in visualizing the particles in follicular locations other than the basal lamina by employing certain procedures in preparing the material for examination by transmission electron microscopy. Treatment of the fixed blocks with tannic acid (Simionescu & Simionescu, 1976) proved to be especially valuable in the preservation of the particulate material. In this report the distribution of particles is described in regions of the follicle enclosed by the granulosa basal lamina. The observations provide morphological evidence for the transfer of particles, possibly VLDL, across the oocyte plasma membrane into the yolk spheres.

METHODS

The hens were obtained from a stock of White Leghorns maintained on a standard diet and 14-h diurnal cycle. Those selected for examination were laying at regular intervals with sequence lengths of at least 6 eggs. They were given a lethal dose of pentobarbitone sodium (Expiral, Abbott Laboratories) at the mid-point of the light phase usually after ovulation had occurred on that day. Ovarian follicles, ranging from 18 to 30 mm in diameter, i.e. 1–5 days preceding ovulation, were removed from the ovary and fixed within 1–2 min of death. In some experiments, the follicles were taken from anaesthetized birds to minimize post-mortem changes. No structural differences were detected so the first method was routinely adopted for ease of operation. A total of 41 follicles from 15 birds was examined. In another experimental series the follicles were removed from the ovary at 1, 5, 7, 10, 15 and 20 min after death in order to follow changes in structure resulting from delayed fixation. A total of 11 follicles from 3 birds was examined in this series.

Two methods of staining the material prior to dehydration were used. Furthermore, the stage in the processing procedure at which the oocyte surface layer plus the granulosa layer were separated from the adjacent theca and yolk mass was a critical factor in the preservation of particular structural conformations; the details are given in each staining method. The solutions were buffered with sodium cacodylate, pH 7·2, and used at room temperature unless otherwise stated.

Tannic acid. In one method, intact follicles were immersed in a solution containing 2·5 % glutaraldehyde, 1·5 % paraformaldehyde, 1 % acrolein and 2 mM $CaCl_2$ in 0·1 M buffer for 18 h. An alternative fixative containing 5 % acrolein and 2·5 % glutaraldehyde was less satisfactory. They were rinsed in 0·2 M buffer for 1 h; during this period, strips of follicle wall, including theca, granulosa and oocyte surface layer were excised and cleaned of most of the adherent yolk. In a second method, the oocytes and granulosa layer were released from the theca (Gilbert, Evans, Perry & Davidson, 1977) and immersed in a modified Karnovsky's solution containing 2·5 % glutaraldehyde, 2 % paraformaldehyde and 2 mM $CaCl_2$ in 0·1 M buffer for 2 h. Strips of the surface layer from the main body and germinal disk region of the stripped follicles were excised and rinsed as before. The material from both treatments was further processed as described by Simionescu & Simionescu (1976). It was postfixed in 2 % osmium tetroxide in 0·1 M buffer for 2 h at 4 °C, cut into 1 × 2 mm strips and rinsed for 1 h in 3 changes of 0·1 M buffer. It was then treated with 1 % tannic acid (Code No. 1764, Mallinckrodt Inc.) in 0·05 M buffer for 0·5 h and rinsed in 1 % sodium sulphate in 0·05 M buffer for 10 min, followed by 0·1 M buffer for 10 min. Specimens not treated with tannic acid were used as controls for the method. After dehydration in ethanol the tissue blocks were infiltrated with epoxypropane and embedded in an Epon/Araldite mixture. Thin sections were mounted on grids coated with Formvar and carbon, and stained for 5 min with lead citrate. They were examined by transmission electron microscopy.

Uranyl acetate. In one method, intact follicles were immersed in the acrolein/glutaraldehyde fixative overnight. They were rinsed in 0·075 M buffer containing 0·2 M sucrose and strips of the surface layer were removed. The strips were postfixed in 1 % osmium tetroxide in veronal acetate buffer, pH 7·2, with 0·2 M sucrose (Caulfield's fixative) for 2 h at 4 °C. After a brief rinse in the buffer, they were stained in 0·5 % uranyl acetate in veronal acetate buffer, pH 5·0, for 1·5 h. In a second method, follicles were stripped of the thecal layers and fixed in modified Karnovsky's solution for 2 h. They were rinsed, postfixed and stained, taking care to avoid rupturing the surface of the specimen. After transfer to 70 % ethanol, strips of the surface layer were excised and cleaned of yolk. The tissue blocks from both treatments were dehydrated and embedded. Thin sections were stained in 1 % uranyl acetate in 50 % ethanol, followed by lead citrate.

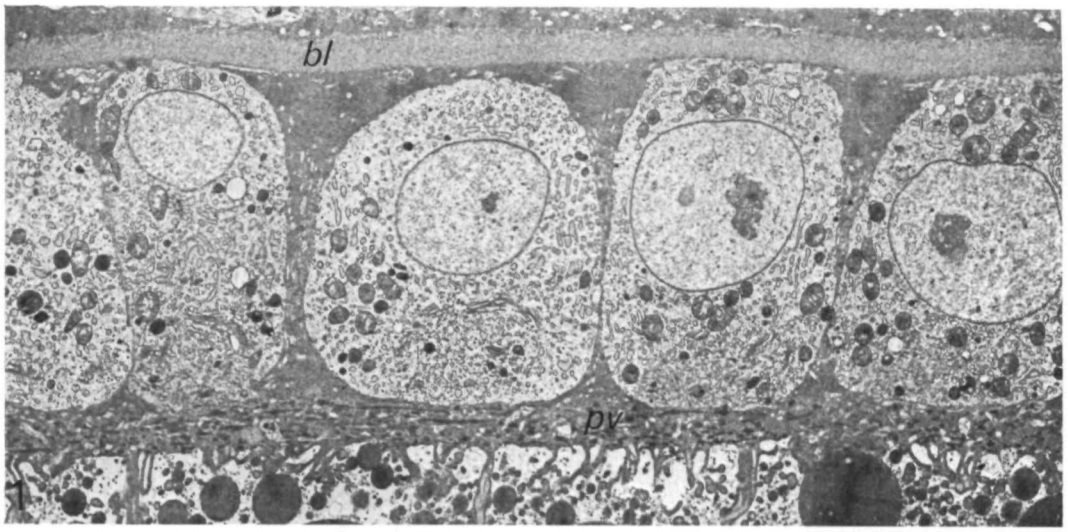
Particle diameters of tannic acid preparations printed at a final magnification of 88 500, were measured using a × 8 graticule magnifier. In each follicular site, 500 particles from 7–17 prints were measured in increments of 0·1 mm. From these data the frequency distributions of particle sizes were analysed and estimates obtained of their mean diameters.

RESULTS

General morphology

The organization of the granulosa and oocyte surface layer of follicles fixed with the theca intact is illustrated in Fig. 1. A basal lamina, 1·0 μm thick and of moderate electron density, surrounded the single cell layer of the granulosa. The large intercellular spaces in this layer were filled with material of high electron density; they also contained granulosa cell microvilli which appeared as light profiles embedded in the dense material. Proximally the perivitelline layer, composed of a loose meshwork of electron-dense fibres, surrounded the oocyte. The oocyte surface was deeply indented with narrow pouches containing granulosa cell macrovilli and oocyte microvilli. Dense material was present throughout the perivitelline space.

An improvement in the structural detail of the oocyte surface layer was obtained in follicles from which the theca had been removed before fixation. This manipulation tended to pull the granulosa cells apart and to stretch out the oocyte surface (Fig. 2). There was a consequent dispersal of the extracellular contents, and possibly some loss of this material during subsequent processing procedures, since it varied in amount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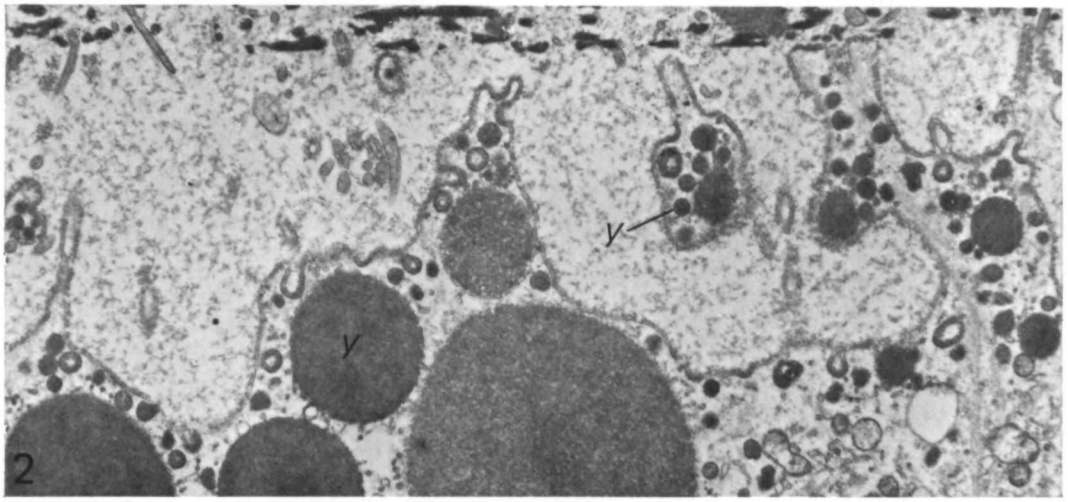
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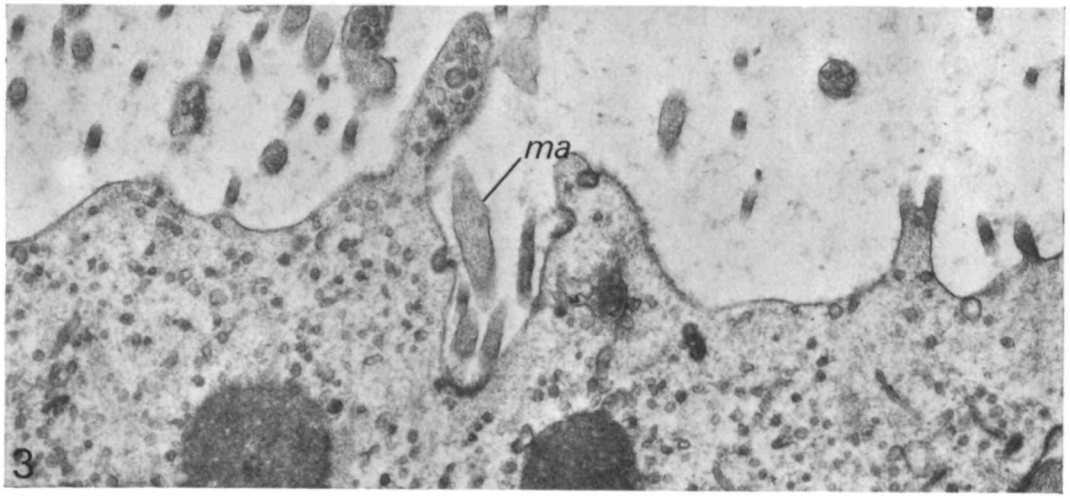
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between specimens. A thin layer of material, however, remained attached to the oocyte surface; it was continuous over the microvilli and in the coated pits distributed along the surface. In the peripheral cytoplasm (Figs. 2, 4) to a depth usually of not more than 1·0 μm, there were large coated vesicles, average diameter 280 nm, and numerous dense bodies of variable dimensions, > 250 nm, the prospective yolk spheres. Other features of the superficial cytoplasm were uncoated vesicles, about 70 nm diameter, mitochondria with sparse cristae, traces of microfilaments and, occasionally, small Golgi complexes. At a deeper level, but sometimes lying close to the oolemma, were the larger, definitive yolk spheres. Unlike the small yolk spheres these were complex bodies, consisting of dense granular masses (see Bellairs, 1964) dispersed in an apparently homogenous matrix. The granular masses tended to be obscured in specimens treated with tannic acid.

Because slight variations in this general pattern were found particularly with regard to the presence of coated vesicles and extracellular material on the oocyte surface, the effects of delayed fixation were investigated. The temperature of the follicles in which the circulation had ceased was slightly less than 38 °C. After 5 min in these conditions the extracellular material was sparse and the coated vesicles were smaller than normal, averaging 125 nm diameter. After 7 min there were few coated vesicles (Fig. 3) and at later times they were virtually absent. There was a corresponding decrease in the number of small yolk spheres. Simultaneously, numerous uncoated vesicles, 70 nm diameter, appeared in the superficial cytoplasm.

Particulate structures

When tannic acid-treated specimens were viewed at a higher magnification the dense material described in the preceding section was seen to consist of particles. These were circular or ovoid structures of moderate electron density, bounded by a narrow rim of higher density. The particles were dispersed throughout the basal lamina of all preparations. They formed tightly packed arrays in the intergranulosa (Fig. 5) and perivitelline spaces of specimens that had been immersed in the fixative

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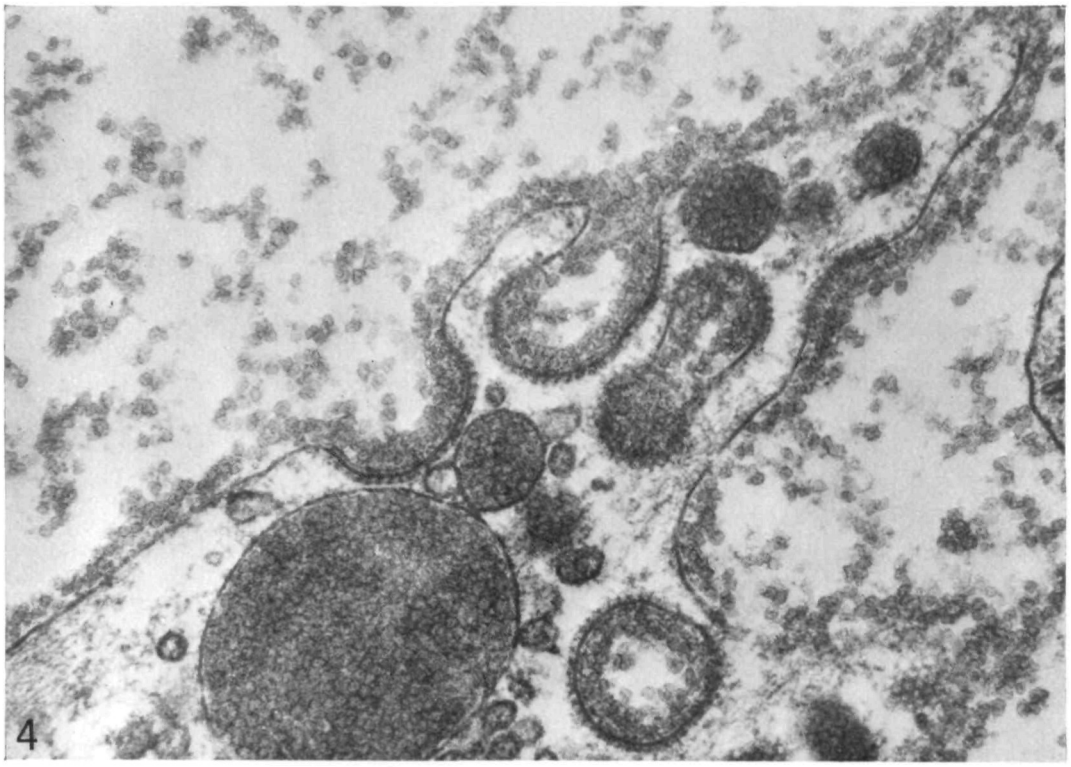
Fig. 1. Radial section through the granulosa layer of a 2-day preovulatory follicle. The intercellular spaces are filled with dense material. The granulosa basal lamina (*bl*) appears as a lighter zone adjacent to the granulosa; the fibres of the perivitelline layer (*pv*) are seen as denser bodies embedded in the perivitelline material. Follicle fixed with the theca intact. × 3500.

Fig. 2. In a follicle stripped of the thecal coverings and fixed 2 min post-mortem, the material in the perivitelline space is dispersed. The irregular surface of the oocyte is covered with fuzzy material and indented with pinocytotic pits. In the superficial cytoplasm are several pinocytotic vesicles and numerous yolk spheres of various dimensions (*y*). × 7500.

Fig. 3. In a follicle stripped and fixed 7 min post-mortem there is little extracellular material and few pinocytotic vesicles or small yolk spheres. The cytoplasm contains numerous smooth, 70-nm, vesicles. *ma*, granulosa cell macrovillus. × 22000.

All preparations were treated with tannic acid.



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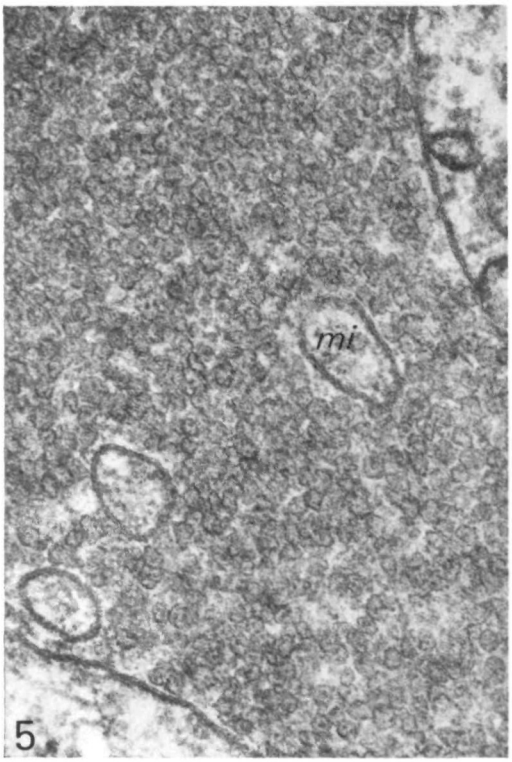

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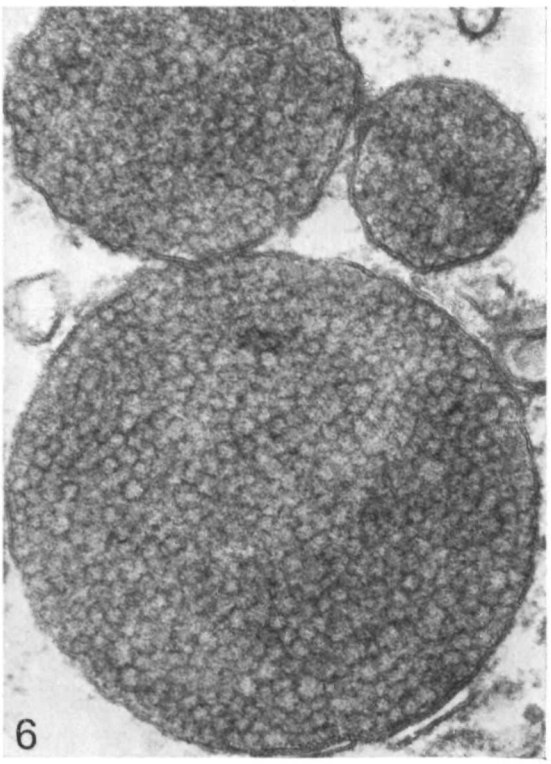

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with the theca intact. In the stripped specimens they were scattered in the extracellular spaces and formed the compact layer on the oocyte surface and in the coated pits and vesicles (Fig. 4). The nascent yolk spheres had a particulate structure of similar morphology to that in the extracellular space (cf. Figs. 5, 6) but of poorer definition, presumably as a result of the tighter packing of the particles. Depending on whether it was adequately penetrated by the processing reagents, the matrix of the definitive yolk spheres was also observed to have a particulate structure. The particle size ranged from 15 to 40 nm with a mean diameter of 27 nm (Table 1). Analysis of the frequency distributions showed small differences between the sites that may be statistically significant, but what was striking about the results was the remarkable similarity between the distributions at different sites.

Table 1. *Percentage frequency distributions of particle diameters in sites enclosed by the granulosa basal lamina of the ovarian follicle of Gallus domesticus*

Site	Diameter size class, nm					Mean diameter, nm
	15–20	20–25	25–30	30–35	35–40	
Basal lamina	4·6	31·2	44·4	16·4	3·4	26·8
Extracellular space	3·6	29·0	46·4	18·4	2·6	27·0
Oolemma	4·8	33·2	43·6	16·6	1·8	26·5
Oolemmal pits/vesicles	4·9	34·9	47·5	11·7	1·0	26·6
Yolk spheres	2·2	29·4	44·2	22·4	1·8	27·3

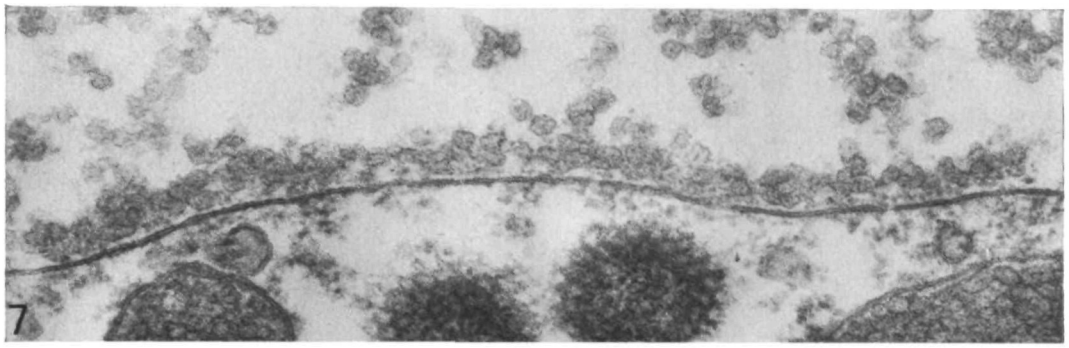
A comparison of the plasma membranes of yolk- and non-yolk-forming regions of the stripped follicles revealed differences in the distribution of adherent particles. A fairly continuous and approximately double layer of particles covered the oolemma over the yellow yolk, i.e. the major part of the oocyte (Fig. 7). Whilst many of the particles were attached to each other, a gap of 5–7 nm usually separated them from the outer leaflet of the trilamellar plasma membrane. There were fewer particles on the oolemma in the germinal disk region, characterized by microvilli overlying white yolk, and almost none on the oolemma over the germinal vesicle (Fig. 8). A corresponding diminution in the number and size of coated vesicles has been described in the germinal disk (Perry *et al.* 1978*b*). The plasma membranes of the granulosa cells were, in general, devoid of particles; even in specimens containing a heavy concentration of this material in the intercellular space, few particles appeared to adhere to the plasmalemma or to the coated vesicles (Fig. 9). In areas of intimate association between the

Figs. 4–6. Extra- and intracellular particulate material.

Fig. 4. Particles are scattered in the perivitelline space (see Fig. 2). They form a thick layer on the oolemma and in the bristle-coated pits and vesicles. × 57000.

Fig. 5. The compact material between the granulosa cells of follicles fixed with the theca intact (see Fig. 1) contains abundant particles. Granulosa cell microvillus (*mi*). × 88500.

Fig. 6. Tightly packed particles of similar morphology to those found extracellularly form yolk spheres bounded by trilamellar membranes. × 88500.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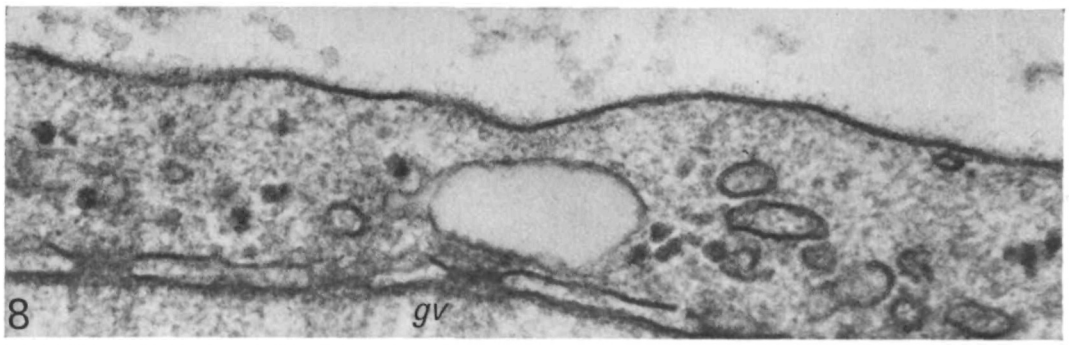
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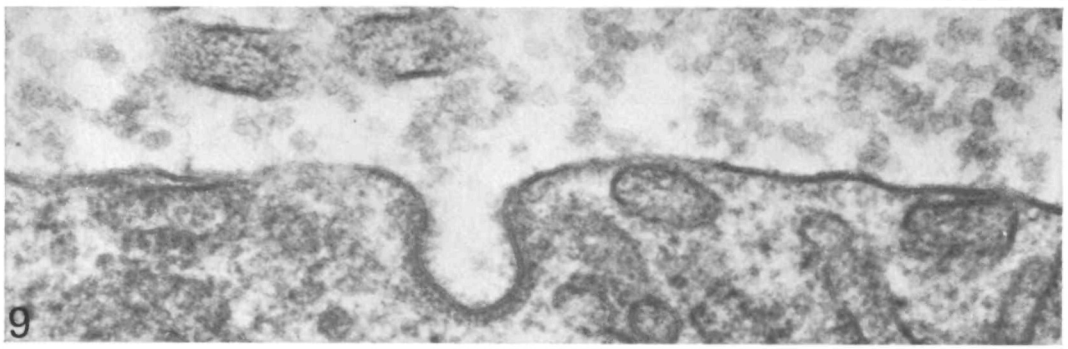
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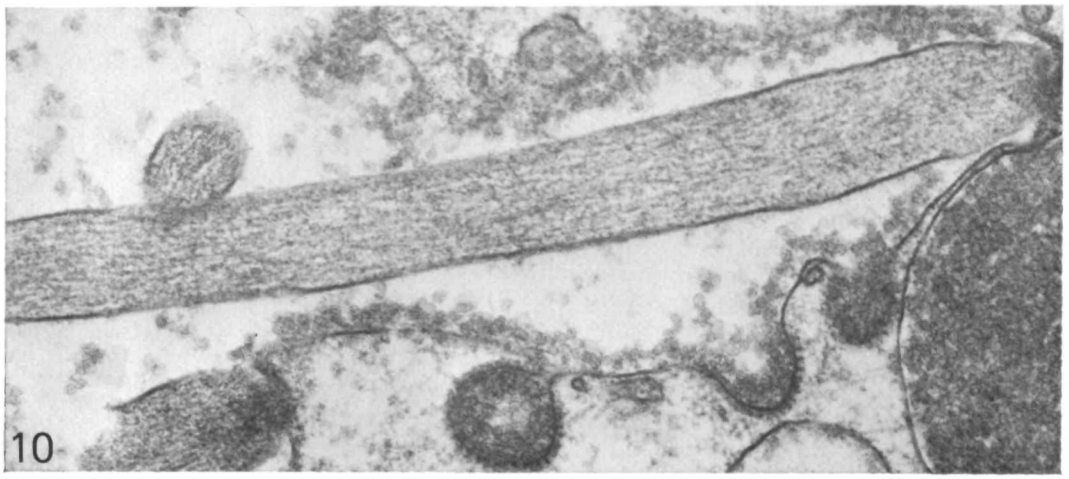
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granulosa cells and the oocyte, where macrovilli extended into the infoldings of the oocyte surface, there was little evidence of particles on the granulosa cells (Fig. 10).

The following sequence, illustrated in Figs. 11–18, is proposed for the packaging of the particles into pinocytotic vesicles and their subsequent transfer to the ooplasm. The vesicles first appeared in the oocyte surface as shallow depressions which gradually deepened and eventually were pinched off from the surface. They were complex structures and ranged from 200 to 350 nm in diameter, taking the outer leaflet of the trilamellar membrane as the boundary. On their cytoplasmic aspect a coat of bristle-like projections, 20 nm thick, represented cross-sections through the polyhedral lattice characteristic of coated vesicles in many cell types (Kanaseki & Kadota, 1969; Pearse, 1976). A row of 10-nm granules formed a complementary layer on the external, or luminal aspect of the bounding membrane. These granules were most clearly seen in specimens lacking well-preserved particles (Fig. 19). Lining the coated pits and vesicles was a double layer of particles (Figs. 11–14); in some cases loosely packed particles were observed in the vesicular lumen. These large coated vesicles were invariably located immediately beneath the oolemma and it is likely that many apparent vesicular structures (Fig. 14) were still connected by a narrow isthmus with the extracellular space. A few small (70–100 nm) coated vesicles were occasionally observed at a deeper level in the ooplasm. Once the vesicles became detached from the oolemma the vesicle wall appeared to collapse into the lumen to form crescentic bodies with bristles coating all but the inner, concave surface (Fig. 15). Other images that were interpreted as transition stages between coated vesicles and nascent yolk spheres were spherical coated bodies with a particulate substructure and similar bodies partially denuded of their bristle coating (Fig. 16). These transition bodies were comparatively rare. The nascent yolk spheres were bounded by a trilamellar membrane (Fig. 17). The presence of pentalamellar membrane structures between apposed spheres indicated that coalescence of these bodies into larger spheres (Fig. 18) was preceded by a process of membrane fusion.

It was found that differences in procedure caused a loss either of the particles as a whole or in their definition. In specimens subjected to delays in fixation the layer of particles on the oolemma had disappeared after 7–10 min, although a few remained in the sparse coated pits (Fig. 20). To visualize particles on the oolemma of uranyl

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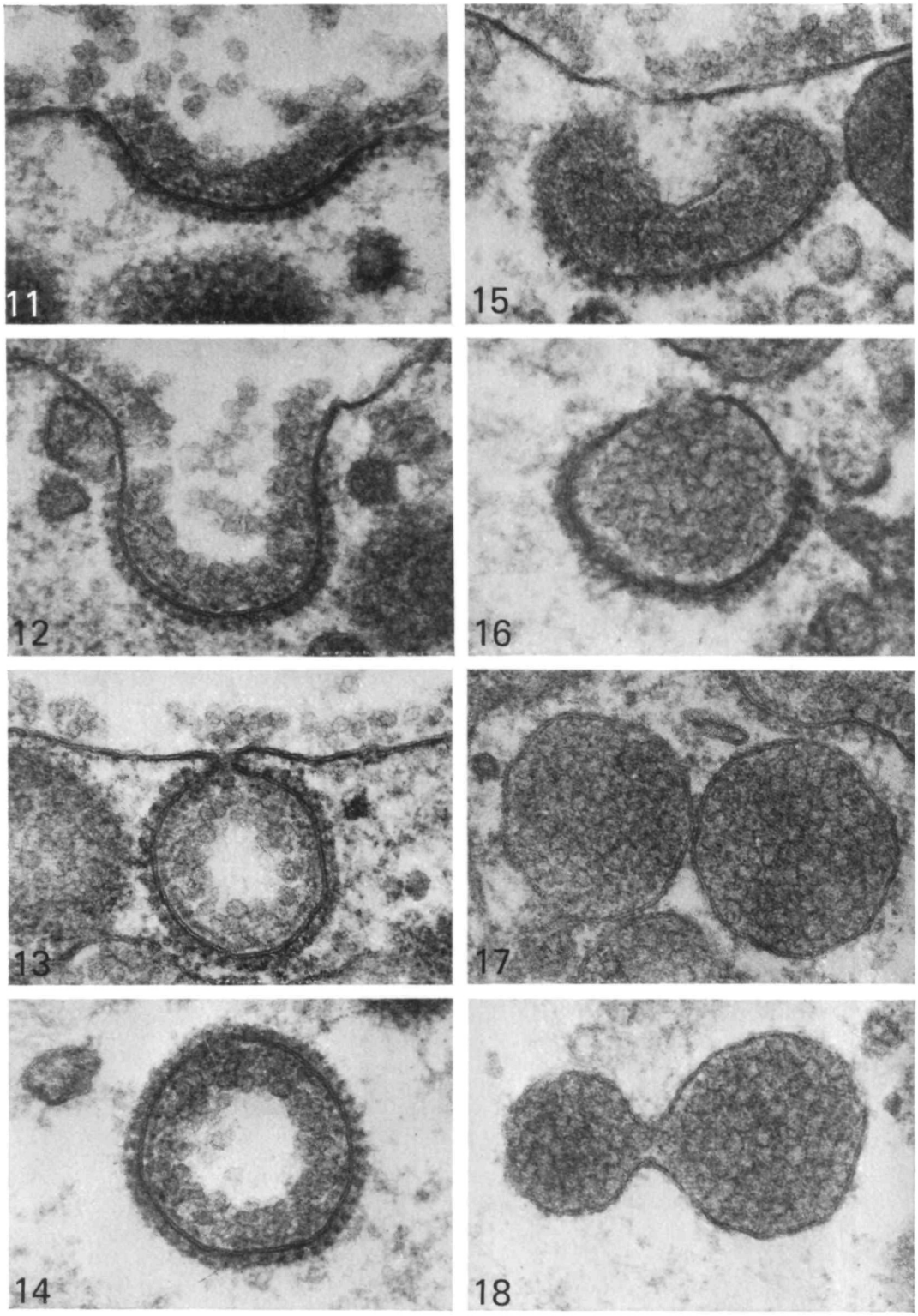
Figs. 7–10. Differential distribution of particles on the plasma membranes. Stripped follicles treated with tannic acid.

Fig. 7. The oolemma of the yellow yolk region of the oocyte is covered by a double layer of particles which are attached to each other but are generally separated by a gap from the external leaflet of the plasma membrane. × 88 500.

Fig. 8. The oolemma of the germinal vesicle (*gv*) region of the oocyte is almost devoid of particles. × 88 500.

Fig. 9. Few particles adhere to the plasma membrane of the granulosa cell or to the membrane in the coated pit although they are abundant in the intercellular space. × 88 500.

Fig. 10. In contrast to the nearby oolemma, the surface of a granulosa cell macrovillus extending into a pouch in the oocyte is free of particles. × 51 250.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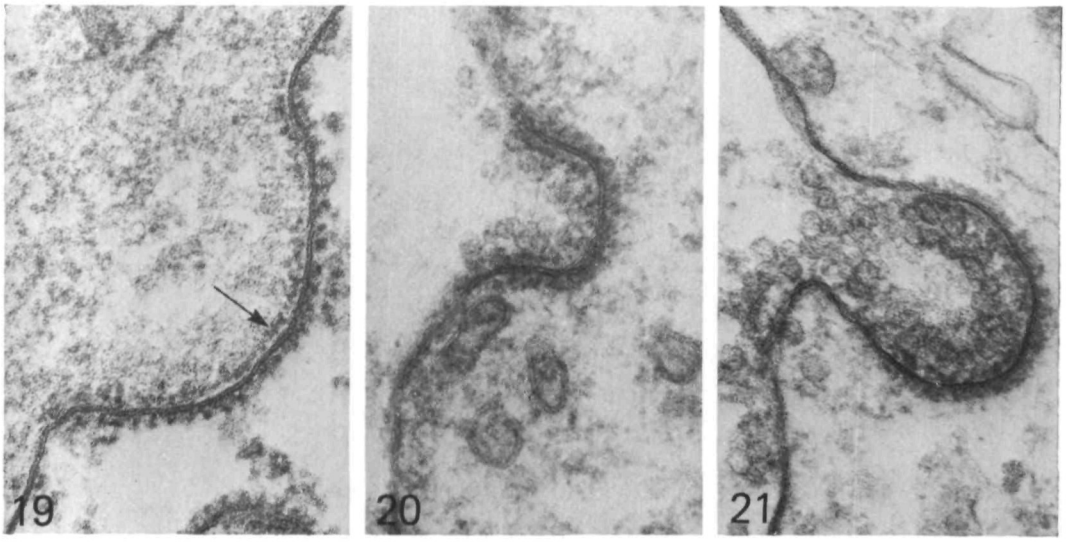

Figs. 11–18. Schematic series in the endocytosis of particles to form yolk spheres. Stripped follicles treated with tannic acid. × 106 500.

Fig. 11. A shallow invagination of the oolemma is coated on its cytoplasmic surface with a layer of bristle-like structures; on its external surface are traces of a layer of dense granules (see Fig. 19) beneath the double layer of particles.

Fig. 12. A deeper coated pit contains several free particles in addition to those adhering to the plasma membrane.

Fig. 13. Final stage in the formation of a coated vesicle prior to fusion of opposed membranes at the neck of the invagination.

Fig. 14. A coated vesicle which is possibly still attached to the oolemma.

Fig. 15. A crescentic body with a cytoplasmic coat of bristles on its convex surface. Such images suggest a collapse of the lumen following detachment of the vesicle from the cell surface.

Fig. 16. A particulate body partially denuded of its cytoplasmic coat.

Fig. 17. Nascent yolk spheres.

Fig. 18. Fusion of nascent yolk spheres.

Figs. 19–21. Aspects of coated pit structure after alternative preparatory methods. × 106 500.

Fig. 19. A row of dense granules (arrowed) on the external surface of an oolemmal indentation coincides with the cytoplasmic coating of bristles. Follicle fixed with the theca intact, dissected, then stained *en bloc* with uranyl acetate.

Fig. 20. Particles in one of the few remaining coated pits of a follicle fixed 7 min post-mortem. Tannic acid.

Fig. 21. Particles in a coated pit of a stripped follicle, fixed, then stained with uranyl acetate and further dissected at the dehydration stage.

acetate-stained preparations it was necessary to keep the stripped follicles intact until they were in the lower alcohols. Particles were present in the oocyte coated pits (Fig. 21) and formed a fragmentary layer on the remainder of the oolemma. The nascent yolk spheres were granular rather than particulate in structure, but tightly packed particles were detected in some definitive yolk spheres. Omission of either tannic acid or uranyl acetate from the processing schedule resulted in a complete loss of particle definition.

DISCUSSION

In this study, 27-nm particles similar to those in the basal lamina (Perry *et al.* 1978*a*, *b*) have been demonstrated in the extracellular spaces of the oocyte and granulosa and in various structures within the oocyte at the final stage of growth. While it is recognized that they are based on static images, the observations suggest that the particles are transferred from the granulosa basal lamina to the oocyte surface via the intergranulosa cell channels. Thence they are packaged in large, coated vesicles which, after passing into the superficial cytoplasm, rapidly lose their cytoplasmic coats to form the nascent yolk spheres. Successive fusion of these bodies gives rise to larger yolk spheres in which there is an apparent segregation of granular material to form discrete masses dispersed in a matrix of compact particles. At a deeper level in the oocyte, where the organization of the yolk is more complex, clumps of ring-like particles, 25 nm in diameter, have been described in the continuous phase of the yolk whereas dense particles, 3–6 nm diameter, comprise the granules, occurring either as free bodies or contained within yolk spheres (Bellairs, 1964; Bellairs, Backhouse & Evans, 1972).

It seems likely that the particles described in this report are mainly VLDL. It has previously been shown (Evans *et al.* 1979) that the isolated basal lamina of the granulosa in the hen's ovarian follicle contains VLDL which is chemically identical with plasma VLDL and is present in concentrations comparable with those in yolk. Elution of the VLDL from the basal lamina was accompanied by a loss of particles with a modal diameter of 25–30 nm, subsequent perfusion of the lamina with plasma VLDL resulting in the reappearance of particles of similar morphology. Some native particles of smaller diameter were considered to be LDL (low density lipoprotein). The particle size determinations in our studies corresponded with those given for egg yolk VLDL (Nichols, Forte & Coggiola, 1969) and for the ring-like particles in the continuous phase of yolk, considered to consist in part of the low density lipoprotein fraction (Bellairs *et al.* 1972). The present observations therefore lend support to the proposals of earlier investigators (Hillyard *et al.* 1972; Gornall & Kuksis, 1973; Holdsworth *et al.* 1974) for the direct transfer of VLDL from the blood to the oocyte. This route of entry for lipids is in marked contrast to that occurring in other tissues which normally absorb the catabolic products of VLDL and chylomicrons as fatty acids (Scow, Hamosh, Blanchette-Mackie & Evans, 1972). Moreover, as pointed out by Holdsworth *et al.* (1974) and confirmed by our observations, the ooplasm contains little by way of cytomembrane systems for the synthesis of

VLDL in quantity. With regard to the localization of the high density lipoprotein (lipovitellin), this component, together with the yolk phosphoprotein (phosvitin), has been identified with the 3–6 nm particles in the yolk granules (Bellairs *et al.* 1972).

The requirement for uranyl acetate or tannic acid in the processing procedures for the visualization of the particles poses the question of how these reagents function in the preservation of lipoprotein particles. Both reagents are effective in enhancing the contrast of membranes, by stabilizing phospholipids in general in the case of uranyl acetate (Silva, Santos Mota, Melo & Carvalho Guerra, 1971) and phosphatidyl choline in particular in the case of tannic acid (Kalina & Pease, 1977). A postulated model for circulating VLDL particles consists of a core of neutral lipids enclosed in a shell of proteins, cholesterol and phospholipids (see Jackson, Morrisett & Gotto, 1976). Phosphatidyl choline comprises a substantial proportion of the phospholipids of hen's plasma and egg yolk VLDL (Gornall & Kuksis, 1973). The outer components of lipoproteins have been estimated to occupy a monolayer, 2·15 nm in thickness (Sata, Havel & Jones, 1972) and a similar value, 2·5–3·0 nm, has been given for the fragmentary surface film observed on sectioned chylomicrons (Blanchette-Mackie & Scow, 1973). It is conceivable that the unilamellar membrane of the particles under investigation represents, at least in part, the phospholipids. The images obtained with uranyl acetate were invariably inferior to those obtained with tannic acid, a result which may be partially attributed to the extractive effects of the former compound on some tissue components. Possibly also, tannic acid selectively 'stains' the limiting membrane in contrast to the surrounding material, enabling the circular profiles of particles to be defined in areas of compacted material, e.g. the nascent yolk spheres, which otherwise appear homogeneous. Potassium ferricyanide, which also has an affinity for membranes, has been employed recently to demonstrate lipoprotein particles in fixed and section preparations (Vermeer, De Bruijn, Van Gent & De Winter, 1978).

The differential distribution of particles on the cell surfaces provides morphological evidence for the selective binding of particles to the oolemma over the yellow yolk mass. Other evidence in support of this observation was obtained from a preparation stained with ruthenium red by the method of Luft (1971). As compared with the 8-nm layer on the granulosa cells, we noted that the oolemma was covered with a dense, 36-nm layer containing poorly defined particles. It is well established that during endocytosis some macromolecules are adsorbed to the plasma membrane by means of specific receptors thus permitting their selective uptake and accumulation from the extracellular fluid, whereas other molecules are engulfed in the fluid phase of the endocytotic vesicle (see Silverstein, Steinmann & Cohn, 1977). In respect of lipoproteins, binding sites have been demonstrated for the phosvitin–lipovitellin complex on hen oocytes (Yusko & Roth, 1976) and for LDL on human fibroblasts (Anderson, Goldstein & Brown, 1976). From the present investigation it seems reasonable to assume that specific receptors may also exist on the hen's oocyte for VLDL. In contrast to the sites for immunoglobulins on the hen's oocyte (Roth, Cutting & Atlas, 1976) and for LDL on fibroblasts which are mainly confined to bristle-coated regions of the plasma membrane, it would appear that the putative

sites for VLDL are uniformly distributed on the oolemma. The abundance of particles in the basal lamina and in the space around the granulosa cells and oocyte indicates that another mechanism, in addition to the selectivity of the oolemma, is involved in concentrating the yolk precursors in the follicle. This mechanism is probably operating at the level of the theca interna since a cursory examination has shown particles to be more numerous in this region than in the theca externa.

The second stage in the absorption of the particles is their enclosure in coated vesicles. While the formation of coated vesicles is a device adopted by various cell types for the selective uptake of proteins and is well exemplified by the yolky oocytes of insects (Roth & Porter, 1964; Roth *et al.* 1976) and amphibians (Wallace & Dumont, 1968), in birds these vesicles attain considerable dimensions, some 2–3 times larger than those found in other cell types (Perry *et al.* 1978*a*, *b*). The fact that particles were present in the 125-nm coated vesicles of oocytes subjected to a delay in fixation, as well as in the 280-nm coated vesicles of rapidly fixed oocytes, indicates that their exceptional size is an adaptation for the absorption of large quantities of particles. However, it is significant that among oviparous animals only the eggs of birds are known to contain a low density lipoprotein fraction (Cook & Martin, 1969). Thus a qualitative as well as a quantitative relationship may exist between coated vesicle size and the size of the molecular species undergoing endocytosis. The value of 85 nm quoted by Woods, Woodward & Roth (1978) for the diameter of coated vesicles isolated from hen oocytes is less than our estimate. This discrepancy between coated vesicles *in vitro* and *in situ* may be explained by the rapidity of the post-mortem changes in vesicle morphology. In the context of coated vesicle formation, the localization of dense granules on the coated invaginations of the oolemma may be related to the mechanism envisaged by Ockleford (1976) whereby a lateral aggregation of components in the plane of the membrane would induce its inward curvature. Whether the granules represent an integral part of the membrane or adsorbed molecules, other than VLDL-like particles, remains to be elucidated.

The fate of the coated vesicles following their detachment from the oolemma parallels that for the vesicles of mosquito oocytes (Roth & Porter, 1964) where the loss of the cytoplasmic coat precedes their coalescence into yolk granules. The events of coat loss and nascent granule fusion are probably rapid, since intermediate forms were comparatively rare. The time scale of the events of adsorptive endocytosis in the hen's oocyte can be roughly assessed from observations on specimens fixed at various post-mortem intervals. These suggest that once the blood pressure in the follicle drops, pinocytsosis proceeds for about 5 min until the extracellular material is depleted, thereafter the remainder of the vesicles and nascent yolk spheres continue to form larger spheres for a period of 5–10 min. If this interpretation is correct then the duration of the entire process of transfer of particles from the perivitelline space to the yolk spheres would broadly correspond with the precise estimates (Anderson, Brown & Goldstein, 1977) for the transfer of LDL to the lysosomal system.

While the major part of the oocyte surface is actively engaged in the endocytosis of yolk precursors in the rapid growth phase, the surface of the germinal disk is relatively quiescent, especially in the 1–2 days preceding ovulation (Perry *et al.*

1978*b*). The consequent diminution of yolk formation in the germinal disk clearly has implications in respect of the events of fertilization and embryogenesis. An abundance of large yolk spheres in the cytoplasm of this region would physically impede the migration of, for example, the sperm and pronuclei, and the growth of cleavage furrows in the blastodisk.

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